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NUTRITION REVIEWS

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NUTRITION REVIEWS

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PROTEIN QUALITY

It is generally stated that it is possible to estimate the biologic value of a protein from a chemical analysis of its constituent amino acids. When sufficient amounts of the essential amino acids are found to be present, the protein food usually is considered to be a first grade protein source.

It has been demonstrated that a mixture of the essential amino acids can be utilized even by human subjects under certain experimental conditions, and the majority of researchers seem to be of the opinion that the end products of the protein digestion in the intestinal tract are amino acids or small peptides. However, there are several investigations which indicate that completely hydrolyzed proteins are nutritionally inferior to unhydrolyzed native proteins. As an example of such experiments D. W. Woolley's work on strepogenin (*J. Biol. Chem.* **162,** *383 (1946)*; **172,** *71 (1948)*) may be mentioned. Furthermore, P. R. Cannon's (*Porter Lectures, Series 14 (1950)*) experiments on the importance of the time factor are also of interest. He showed that under certain experimental conditions it was necessary to give all the essential amino acids at the same feeding, in order to have them used efficiently.

If the enzymatic hydrolysis of proteins in the intestinal tract were comparable with a chemical hydrolysis of pure proteins, we should certainly not have all the essential amino acids available for resorption at the same time, since complete hydrolysis of protein usually takes a rather long time. On the other hand, when the *end products* in the enzymatic process are peptides of relatively high molecular weight containing a complex mixture of amino acids, these amino acids will be available at the same time provided that such big peptides are absorbed and that they can be utilized without further hydrolysis or hydrolyzed after passing through the intestinal wall. It is thus at least *possible* that not only the amount and type of amino acids in a certain protein, but also the way in which the protein is physiologically hydrolyzed, may be of nutritional interest.

Apparently several factors act to control the enzymatic digestion of proteins in the intestinal tract and there is a certain amount of indirect evidence that the end products of protein digestion are larger molecules than is usually assumed. In the following, I want to discuss some results from our laboratory, which we interpret in that way, and also some experimental facts indicating that large peptides may have quite specific properties of nutritional interest (O. Mellander, *Acta Soc. Med. Upsal.* **52,** *107 (1947)*).

So far, we have been interested mainly in protein digestion and utilization in infants during the first year of life, when the food protein consists almost exclusively of milk proteins and the mode of living is rather standardized.

It is easy to demonstrate that the most characteristic of the milk proteins, casein, when digested *in vitro* by the proteolytic enzymes of the intestinal tract, gives a considerable residue of high molecular peptides resistant to further action of proteolytic enzymes. This resistance is probably due to the fact that these peptides are phosphorylated, since dephosphorylation by phosphatases eliminates the resistance to proteolytic enzymes. We have worked out methods for preparing such peptides in kilogram quantities from cow's milk casein and have prepared them on a smaller scale also from human and goat's milk casein. The most interesting property of these peptides is their tendency to form complexes with various other substances. As a matter

of fact, they seem to behave as effective chelating agents and may be of biologic importance due to this fact. The calcium complex, for example, contains about 10 per cent of calcium. This calcium-phospho peptide is highly soluble to about 50 g. per 100 ml. of solution. Calcium in this form is absorbed from the intestinal tract of infants to an extent of 70 per cent and perhaps more. When given to rachitic animals, for example chicks, calcium in this form seems to be utilized, even if vitamin D in curative dose is not given. The calcium-phospho peptide was also tested in a few cases of human rickets (O. Mellander, *Acta Soc. Med. Upsal.* **55,** *247 (1950)*).

When this calcium-phospho peptide is given intravenously to rabbits, the calcium level in the serum can be raised from 11 to 12 mg. per cent to 23 to 26 mg. per cent with no apparent discomfort. When injected into old rabbits, the increase is more pronounced than when young rabbits are used (O. Mellander and B. Isaksson, *Acta Soc. Med. Upsal.* **55,** *239 (1950)*).

The iron-peptide complex containing about 20 per cent of iron was given in repeated intravenous injections to rabbits up to a total of 1.4 g. of iron. Iron in this form given by mouth to human subjects caused a rise in the serum iron, thus indicating it was absorbed.

Metals other than calcium and iron form complexes with the large peptides of casein, but not all of them are as water-soluble as the two mentioned. However, these experiments (*Acta Soc. Med. Upsal.* **55,** *239, 247 (1950)*) seem to indicate that high molecular peptides, formed when casein is digested with proteolytic enzymes, may act as vehicles for certain metals and that the calcium and iron peptides can be used intravenously without causing any anaphylactic reactions. To what extent the peptides are used for the physiologic transport of metals through the intestinal wall is an interesting problem, so far not solved. The high utilization of, for instance, the calcium in milk, as compared with inorganic calcium, may be due to the formation of such a peptide.

The peptides mentioned are enzyme-resistant. Other food proteins than casein do not, so far as we know, form enzyme-resistant peptides, when digested *in vitro*. *In vivo* there are perhaps factors leading to an inhibition or retardation of the enzymatic hydrolysis. It should be emphasized that the substrate for protein digestion in the intestinal tract is of course always a mixture of proteins. In milk, for example, three caseins and many albumins and globulins are present. The protein digestion *in vivo* therefore may not be compared with *in vitro* experiments using pure isolated proteins as substrate. When for instance a casein solution is mixed with an egg albumin solution, the rate and degree of hydrolysis is lower than when the casein is digested separately under similar conditions. This fact is difficult to interpret. H. Lineweaver and C. W. Murray (*J. Biol. Chem.* **171,** *565 (1947)*) think that the antitryptic activity of ovomucoid is the reason. Interaction between peptides released during the enzymatic hydrolysis may also be taken into consideration. Also, the presence of certain carbohydrates retards protein digestion.

That a nutritionally adequate protein under physiologic conditions can be less easily digested than a nutritionally inferior protein is clear from the following experiment on human versus cow's milk proteins: Human or cow's milk was fed to young infants and the degree of hydrolysis was followed using the Van Slyke determination of amino nitrogen. The proteins of human milk are found to be less easily digested in the infant's stomach than cow's milk proteins. This is true also with regard to the isolated casein complex as well as when tryptic digestion is concerned. Thus, from this point of view the nutritional value of a protein does not depend on its hydrolyzing in the intestinal tract. The contrary might be true.

To sum up, our working hypothesis is that proteins are utilized partly as large peptides. Experimental evidence is given for the formation of such peptides, when certain milk proteins are enzymatically digested. It is probable that certain mixtures of proteins are less completely hydrolyzed than pure proteins. Large peptides as such may be of nutritional importance, because of the time factor in protein utilization and because they may combine with other nutrients, for example calcium and iron, and favor their absorption.

In the evaluation of vegetable protein combinations of high nutritional value to use as substitutes for animal protein, it is possible that not only the amino acid content has to be taken into consideration, but also the type of peptides formed physiologically. It is possible that the larger the peptides of a certain protein which an organism is able to utilize, the better the nutritional quality of the protein.

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PHYTATES AND RADIOACTIVE CALCIUM UPTAKE IN CHILDREN

Many investigators have studied the influence of inositol hexaphosphates (phytic acid) on the uptake of calcium by human subjects. This form of phosphate represents an appreciable amount of the total phosphorus in diets that are rich in the cereal grains. Inositol hexaphosphate forms insoluble complexes with calcium, and in this manner it can affect the extent to which food calcium can be absorbed.

In a recent study the isotope technic was employed to get quantitative information regarding the extent to which food phytic phosphate can tie up food calcium and thus prevent its absorption (F. Bronner, R. S. Harris, C. J. Maletskos, and C. E. Benda, *J. Nutrition* **54,** *523 (1954)*). The effect of a phytate-rich cereal (oatmeal) on the uptake of calcium was compared with that of a phytate-free cereal (farina). Milk, commonly eaten in conjunction with cooked breakfast cereals, was used as the main calcium source in the meals. The effect of food phytates (oatmeal) was also compared with that of soluble sodium phytate which was added to the farina.

Nineteen adolescent boys, of inadequate intelligence but otherwise normal, who were in a state school under a uniform nutritional condition, volunteered for the experiments. Each subject received a multivitamin tablet and one quart of milk daily for one month prior to the experiment and until it was concluded. All subjects received two test meals three weeks apart. The three-week interval was required to reduce the tissue and excretory levels of radioactive calcium from previous test meals to negligible amounts. The test meals were so formulated that they contained just sufficient calcium (mainly from milk) to combine with all of the food phytate to form pentacalcium phytate. The isotopic calcium (Ca^{45}) used as the tracer (0.85 microcurie of calcium chloride ($Ca^{45}Cl_2$)) was thoroughly mixed with the milk and cereal in the test meals.

The phytate naturally present in oatmeal was less reactive with food calcium than the sodium phytate added to the farina test meal. In the latter case 34 per cent of the food calcium entered the body, whereas with the oatmeal test meal 55 per cent of the food calcium entered the body. In both test meals the calcium content of the meal was between 80 and 90 mg.

The finding that the subjects fed sodium phytate actually took up 34 per cent of the calcium ingested indicates that not all of the calcium reacted with the phytate to form pentacalcium phytate, that the calcium

of pentacalcium phytate was partially available, or that compounds other than pentaphytates were formed. The second explanation is substantiated in experimental studies with the chick in which it has been demonstrated that radioactive phytate phosphorus is absorbed to some extent, presumably as the result of the action of the enzyme phytase in the intestine (*Nutrition Reviews* **9,** *111 (1951)*).

Calculated from the tracer content of serum, the Ca^{45} uptake by the boys on the oatmeal breakfast was 74 per cent as great as that of the boys on the farina breakfast. Similarly, the farina-plus-sodium-phytate breakfast resulted in a calcium uptake 45 per cent that of the farina alone. These differences were statistically significant and established that less calcium enters the body when phytate-containing meals are eaten than when phytate-free meals are ingested. A similar conclusion was reached by R. A. McCance and E. M. Widdowson in calcium balance studies in human subjects (*Brit. J. Nutrition* **2,** *401 (1948)*). Bronner and his associates point out, however, that the food calcium rendered unavailable by food phytates in their studies was so small (15 mg. of the 83 to 90 mg. in the meals) that their effects cannot be considered of major significance in the usual United States diets.

THE FATE OF HYDROCORTISONE IN MAN

Using hydrocortisone labeled in ring A with C^{14}, L. Hellman *et al.* (*J. Clin. Invest.* **33,** *1106 (1954)*) studied the excretion of this compound in 4 patients: 2 suffering from breast cancer, one of whom had a bilateral excision of the ovaries and adrenalectomy, one suffering from chronic lymphatic leukemia, and a fourth with multiple benign lipomata. Tracer doses of the labeled hydrocortisone were used in each instance, but in 2 cases this was combined with a large dose of the hormone (100 mg.). The hormone was administered intravenously.

Negligible amounts of C^{14} were found in the expired-air carbon dioxide so it seemed safe to conclude that little, if any, of the hormone was completely metabolized in the body. Nor was radioactivity detected in spinal fluid eight hours after infusion of a tracer dose.

Within twenty-four hours after administration between 70 and 80 per cent of the radioactivity was found in the urine, presumably as conjugated metabolites of the hormone. Most of the remainder was excreted in the feces within seventy-two hours.

When a large dose of hormone was administered to the patient with breast cancer with intact adrenals, no change in the rate or quantity of urinary elimination of the C^{14}-labeled hormone was observed.

The patient with breast cancer who had been adrenalectomized had her usual oral cortisone withdrawn two days before the study, and the tracer dose was then administered. Symptoms of adrenal insufficiency occurred, but in spite of this no difference in the rate or quantity of urinary excretion of the labeled hydrocortisone was observed. This same patient was studied with a tracer dose of hydrocortisone during a period when she was receiving the 100 mg. of the hormone intravenously daily. Again, no change in rate or quantity of the labeled cortisone was observed.

Although radioactivity remained in the blood for several hours after hydrocortisone administration, a maximum of 13 per cent was found as circulating unaltered hydrocortisone in one experiment fifteen minutes after the end of the infusion, suggesting that the hormone had been rapidly metabolized. The authors noted that, although the hormone appeared to be rapidly metabolized and moderately rapidly excreted in the urine, the manifestations of hormonal effect

such as eosinopenia and increased nitrogen excretion take some hours to become established after administration of the hormone. It seemed to the authors reasonable to believe therefore that the hormone initiated a "chain of reactions."

These studies clearly point out the rapid conjugation or alteration of hydrocortisone given intravenously to man and the prompt excretion of metabolites in the urine. No evidence was obtained of its complete metabolism to carbon dioxide.

UTILIZATION OF IRON COMPOUNDS IN ENRICHED BREAD

It has been ascertained that a variety of factors condition the availability of iron in the diet as administered in the treatment of hypochromic microcytic anemia. Important in these considerations is the form in which the iron is provided. Greatest utilization has been demonstrated to be associated with iron salts which are most readily soluble and ionizable, and in which the iron occurs in the ferrous state (C. V. Moore, R. Dubach, V. Minnich, and H. K. Roberts, *J. Clin. Invest.* **23,** *755 (1944)*; P. F. Hahn *et al.*, *Am. J. Physiol.* **143,** *191 (1945)*). It would seem important, therefore, that advantage should be taken of this information in the provision of iron in bread enrichment mixtures. Although ferrous sulfate is the salt of choice by this assessment, it is used less in the commercial enrichment of bread than are reduced iron, ferric orthophosphate and sodium ferric pyrophosphate. In a well conceived study of great practical significance R. Steinkamp, Dubach, and Moore (*Arch. Int. Med.* **95,** *181 (1955)*) have demonstrated that iron compounds of the latter group are utilized equally as well as ferrous sulfate in the bread enrichment mixture.

These investigations utilized radioiron, Fe^{59}, which was incorporated into the iron compounds most commonly used for bread enrichment. By this means ferrous sulfate, reduced iron, ferric orthophosphate and sodium ferric pyrophosphate of relatively high specific activity were obtained. Each iron preparation was combined with the vitamins utilized in bread enrichment and added to the sponge for the baking of 19-ounce loaves of bread. The baking process, itself, conformed to those in use in commercial bakeries. The quantity of iron added was the same in all experiments and was 12 mg. of elemental iron per pound of flour. Additional iron was present in other ingredients in the loaf so that the final product contained about 0.03 mg. of iron per gram of bread.

Portions of approximately 100 g. of the enriched bread containing the radioiron compounds were eaten by each of a group of 32 young normal adults. Twenty-six were male medical students and 6 were females. The age range of the group was from 21 to 32 years. The bread was consumed with butter and water in the morning as breakfast, after an overnight fast. The feces of all subjects were collected and the quantity of radioiron unabsorbed determined on daily specimens until a day's specimen contained less than 1 per cent of the amount ingested. In addition, the quantity of Fe^{59} appearing in newly synthesized hemoglobin in blood was determined. After a further period of several weeks each subject participated in one of three control studies. These included (1) ingestion of the same quantity of the Fe^{59} compound without bread or (2) with as much ordinary bread as had been eaten as the radioactive iron-enriched bread, and (3) the identical quantity and type of tagged bread taken with a supplement of 1 g. of ascorbic acid.

When ferrous sulfate was the compound used in the bread formula fed to 11 subjects, from 90 to 102 per cent of the radioiron tag was accounted for in feces and in blood. There was variation from 0.8 to 11.2 per

cent in the amount of tagged iron found to be absorbed and utilized in hemoglobin synthesis, with an average value of about 4.5 per cent. Considerably more iron was utilized when the same quantity of ferrous sulfate in solution and without bread was ingested by 5 subjects. Mean percentage utilization for hemoglobin synthesis in this group was 13 per cent. In other subjects who consumed the labeled ferrous sulfate plus a quantity of commercial bread comparable to the quantity ingested of bread containing the iron tag, there was no difference in utilization of iron, indicating that bread or some component in it inhibits absorption of the metal from the intestinal tract.

Similar experiments were conducted utilizing Fe^{59} as reduced iron powder which was thoroughly mixed in the flour in preparation of the loaf for baking. Iron utilization of 5 persons who consumed this preparation was of the same order as of those who had eaten the bread which contained ferrous sulfate. Two subjects had much greater utilization, with values of 26 and 38 per cent. One of these, a woman, was demonstrated to have low iron stores. In control studies in which the reduced iron was ingested along with commercial bread, iron utilization was like that of the same individuals when the tag was incorporated in the bread at baking.

When ferric orthophosphate and sodium ferric pyrophosphate were added to bread prior to baking, the iron contained was similarly available to these normal young adults and utilization rates varied from 2 to 11 per cent. Two persons who consumed bread containing sodium ferric pyrophosphate were found to incorporate larger quantities of the ingested iron in newly synthesized hemoglobin. Both of these were demonstrated to have low serum iron values and therefore were considered to be iron deficient. Like ferrous sulfate and reduced iron, ferric orthophosphate and sodium ferric pyrophosphate were absorbed at the same rates when taken with commercial bread or incorporated into the loaf prior to baking.

It is abundantly clear from the data presented that no difference exists in the availability from enriched bread of the four radioactive iron preparations which were studied. The authors record similar data on the utilization of labeled ferrous sulfate, ferric orthophosphate and sodium ferric pyrophosphate incorporated in bread and fed to 3 patients with iron deficiency due to blood loss. It is clearly demonstrated that these patients were able to absorb iron quite efficiently from the compounds in enriched bread.

Another interesting and thought-provoking finding is described. This relates to the effect of administration of 1 g. of ascorbic acid on iron absorption from the breads fed. In each instance ingestion of the vitamin immediately after eating the labeled bread produced a markedly enhanced absorption and utilization of iron. Although this effect might be presumed to be due to the reducing action of ascorbic acid, it increased the absorption of iron from breads containing ferrous sulfate and reduced iron just as much as it did that to which ferric salts had been added.

The results of these experiments should be of interest to many groups, but perhaps most importantly to food technologists on whose shoulders lies the responsibility for implementation of bread enrichment programs. They seem to prove quite conclusively that reduced iron, ferric orthophosphate and sodium ferric pyrophosphate may be added to enrichment mixtures with full confidence that these compounds are as readily available and as fully utilized as is ferrous sulfate. Practical technologic considerations have dictated the use of these compounds in the past. It is fortunate that evidence is now available to demonstrate the physiologic effectiveness of these materials.

THE EFFECT OF EATING UPON VITAMIN A ABSORPTION

Some observations on the effect of eating upon the absorption of vitamin A palmitate in man have been described by A. I. Mendeloff (*J. Clin. Invest.* **33,** *1015 (1954)*) and should help both to explain the discrepancies in vitamin A absorption previously recorded and to standardize a vitamin A absorption test. A chance observation that the blood vitamin A concentration rose rapidly during an oral tolerance test immediately following a meal led the author to study the effect of eating upon the test.

Synthetic vitamin A palmitate dissolved in corn oil (10,000 micrograms per milliliter) was administered by mouth or tube in a dose of 10.5 ml. A total of 32 normal, healthy control subjects was studied. In this group it was found that when the vitamin A palmitate was given by mouth during fasting very little rise in blood vitamin A level occurred within four hours. In a second group food was administered after two hours and, although in some instances the curve appeared to be rising somewhat by this time, a rapid rise after feeding occurred. Thirty-two hospitalized patients were then studied during fasting. Again, there was some rise in the blood vitamin A two hours after its administration. The rise in most instances became much more marked subsequent to food given at this time. Three totally gastrectomized subjects showed the same response to food as did the others studied.

When the vitamin A was administered by intraduodenal tube to 6 normal subjects, a failure of significant rise within four hours was again noted, but when food was given a rapid rise occurred in each instance. The relation of this rise to food ingestion occurred whether the meal was taken just prior to or immediately after the administration of vitamin A, or if the tube was left in place while food was eaten.

Sham feeding, in which the subjects masticated a meal but expectorated it without swallowing, gave nearly as great a rise in blood vitamin A after the test dose as a meal which was swallowed three hours after the sham meal. Atropine administered just prior to eating appeared to delay the onset of the rise in blood vitamin A concentration. Finally, the results of experiments which isolated a loop of jejunum with balloons, presumably preventing biliary and pancreatic juices from coming in contact with vitamin A instilled into the loop, suggested that these juices could not account for the rapid rise in vitamin A concentration in the blood following a meal.

The authors concluded that the vitamin A palmitate dissolved in corn oil was poorly absorbed, or was absorbed but lay in the lacteals, until after a meal or after sham feeding. The blood concentration rose dramatically after the meal or after sham feeding whereas it rose little during the fasting state. It was suggested by the authors that the test described would be suitable as a vitamin A tolerance test. In such use as a test, of 106 hospitalized patients 13 showed a flat tolerance curve and all had significant steatorrhea, while there were clinical evidences of poor fat absorption in many.

NUTRITIONAL STATUS OF IOWA SCHOOL CHILDREN

A series of papers concerned with the physical measurements and dietary habits of a large number of Iowa school children has been presented by E. S. Eppright and her co-workers, V. D. Sidwell, P. P. Swanson, and C. Roderuck (*J. Nutrition* **54,** *371, 543, 557 (1954)*). These studies represent a large cross-sectional survey of approximately 1200

students between the ages of 6 and 18. Seven-day dietary records were obtained either from the mothers of the children or the children themselves and the average intakes were calculated from food composition tables (B. K. Watt and A. L. Merrill, *U. S. Dept. Agr. Handbook No. 8 (1950)*). Height, weight, chest breadth, bi-iliac diameter and leg girth were measured. Blood samples were analyzed for hemoglobin, alkaline phosphatase, ascorbic acid and carotenoids.

The physical measurements agreed rather well with previous studies upon Iowa school children (R. O'Brien, M. A. Girshick, and E. P. Hunt, Bureau of Human Nutrition and Home Economics, *U. S. Dept. Agr. Misc. Publ. No. 366 (1941)*; R. L. Jackson and H. G. Kelly, *J. Pediat.* **27,** *215 (1945)*), although at most ages the weights tend to be somewhat greater than they were ten years ago while heights are nearly the same. Overweight was more common in girls than in boys and in older girls than in younger girls. The authors were quite impressed with the irregular increments in growth for boys, but in view of the fact that this was a cross-sectional study, there is no evidence that these were not due to random variations in the samples.

The over-all dietary records show that boys eat more than girls. At equal weights the intake of the boys appears to be about 10 per cent higher than that of the girls. Calorie intakes for both boys and girls were considerably above National Research Council Allowances for those between 6 and 8 years of age, but after 10 years they agreed well with the Recommended Allowances. Calorie intakes of girls remained essentially stationary at about 2400 calories after age 12 (weight about 45 kg.), although maximum weight and height were not reached until about 15 or 16 years of age. Calorie intakes of boys continued to increase until about age 16, when they reached 3400 calories per day. The average intakes of all nutrients were equal to or well above recommended amounts at the younger ages and agreed reasonably well with recommended amounts at other ages except for the low calcium and iron intakes of girls above 10 years of age. Older girls tended to be somewhat low in several other nutrients. Whether the irregular changes in nutrient intakes with successive age groups represent more than sample variation and error in dietary records is problematical. Thiamine, riboflavin and niacin intakes were generally closely related to protein intakes.

In the third paper (*J. Nutrition* **54,** *557 (1954)*) the children were classified according to the dietary records. Group I included those whose diets equaled the NRC Allowances in all nutrients, and group III those with at least one nutrient less than 67 per cent of the recommended intake. Calcium and ascorbic acid were the nutrients most commonly causing this latter classification and iron and riboflavin the next most prominent. It is apparent that the children in group III were smaller by all physical measurements and the intake of all nutrients was also less in group III than in group I. The physical measurement data are not given in tabular form, so it is not possible to calculate the difference in nutrient intakes upon an equal weight basis. An inspection of the curves relating physical measurements to age suggests that the slow growers and small eaters in group III reached the same size as members of group I at 15 years of age. This is interesting in view of the considerable difference in calorie intakes between groups I and III. In the group III girls the calorie intake leveled off at about 2100 calories per day at age 12, while in group I the mean intake was over 3000 calories. The difference in calorie intakes in the boys of groups I and III is about 1000 per day during the same period. It is clear that small children eat less food, but no disadvantage in this smaller intake is demonstrable by the data.

Although the authors do not discuss the

point, the data also show that there is a reasonable correlation between the intakes of all nutrients and the calorie intake within the two groups. A rough inspection of the data shows that even for calcium and ascorbic acid, where the standard deviations of the mean intakes are very high, the intakes are roughly proportional to the calories consumed. The proportions are not the same in the two groups, however. The reviewer estimates that in group I the intake of calcium was about 500 mg. per 1000 calories while in group III it was of the order of 350 mg. In any event, the authors are not able to demonstrate any causative effect of the lower nutrient intakes in group III on physical measurements. Since the intakes of all nutrients are rather closely interrelated, and particularly those of protein, thiamine, riboflavin and niacin, speculation as to a causative effect of any one nutrient is unwarranted.

The biochemical data show, as expected, that the plasma carotenoids and ascorbic acid are related to intake. Hemoglobin levels were not related to iron intakes, however.

There is already a considerable body of information upon the average size of normal American school children. The papers discussed herein contribute to our knowledge of the dietary habits of this group, but they perhaps also emphasize the rather scant yield in fundamental information that can be derived from this type of survey in well-fed population groups. The data can be interpreted as showing that people are different and can be grossly divided into two groups, one of which grows more slowly and apparently makes more efficient use of the food eaten.

The large differences in caloric expenditure suggest that the division might be into relatively athletic and nonathletic groups. The data suggest to the authors that "the diets of Group III were sufficiently low in nutrients to retard growth slightly," but this is speculation. Regardless of the correctness of the speculation, it seems of more importance that there is no evidence indicating that the children in group III were in any way at a disadvantage as compared to those in group I.

Preoccupation with the Recommended Allowances may have unfortunate consequences. Since the intake of all nutrients is correlated with calorie intake, this frame of reference forces approval of high caloric intakes in a group of school children who are perhaps already showing tendencies toward obesity. It might have been more useful simply to investigate the dietary habits of two groups of apparently normal school children who differ in physical characteristics. The nutritionist of the future will obviously have to be more and more concerned with the individual rather than expect all persons to meet a standard pattern of physical development or dietary intake. As the authors suggest, longitudinal studies may offer a more satisfactory approach to many questions which still have to be answered. In any event, it should be emphasized to all in the field of nutrition that the important question is always the health of the individual, not the level of nutrient intake per se.

ANTIBIOTICS IN "OLD" ENVIRONMENT FOR CHICKENS

It is well known that antibiotics in the diet may stimulate the growth of certain animals. This is especially true in swine, poultry and calves, which is the reason for the widespread use of antibiotics in animal feeds (*Nutrition Reviews* **8,** *298 (1950)*; **9,** *359 (1951)*; **10,** *107, 211 (1952)*; **11,** *208 (1953)*; **12,** *108, 166, 336 (1954)*). Reports of growth-stimulating effects of antibiotics in children have appeared, but such reports

need confirmation before they can be accepted (*Ibid.* **12,** *271 (1954)*).

The exact mechanism by which antibiotics stimulate growth is not known, but it appears to be associated with the control of deleterious microorganisms in the environment surrounding the animal (*Nutrition Reviews* **11,** *317, 334 (1953)*; **12,** *166, 244 (1954)*; **13,** *54 (1955)*). Support for this theory is found in the ineffectiveness of antibiotics in stimulating growth of animals raised in a new environment (*Ibid.* **10,** *270 (1952)*). Under the latter circumstances the control animals fed unsupplemented diets grow at a better than normal rate.

P. E. Waibel, O. J. Abbott, C. A. Baumann, and H. R. Bird (*Poultry Sci.* **33,** *1141 (1954)*) tested the growth effect of antibiotics in chicks over a three-year period under a wide variety of short-term experiments. The same battery room was used throughout (presumably used for chickens prior to these experiments but this was not stated). Several different types of nutritionally adequate semipurified and natural-type diets were supplemented with either aureomycin hydrochloride or procaine penicillin G at levels usually considered adequate to give a response in growth (from 25 to 35 mg. of aureomycin or from 2 to 200 mg. of penicillin per kilogram of diet). Each experiment was conducted for only three weeks.

During the first twenty-two months of the experiments the supplemented birds grew better than those on control diets in 66 out of 69 experiments. Responses appeared to be especially large (up to 39 per cent) when the three-week weights of the control chicks not receiving antibiotics were lowest. A small effect of changes in dietary composition was noted, but attempts to improve the basal diet by various supplements failed to eliminate the response to the antibiotics during this early period. Responses obtained with the purified diets were as good as responses with natural diets.

After the first twenty-two months the antibiotics lost their effectiveness. Only 7 positive responses were obtained in 14 experiments over a one-year period, which result would be expected from chance alone. The largest response was only 10 per cent regardless of the type of ration used. Unfortunately only one of the rations used in this last period had been tested previously (in two tests only). Also, only one experiment was made with aureomycin (which gave a positive response). All the other results were obtained with penicillin at levels of from 2 to 15 mg. per kilogram of diet, which were not so high as those used in some of the earlier observations. The authors did not discuss the reason for these differences in technic. Perhaps the use of higher levels of penicillin or the use of other antibiotics would have resulted in positive responses.

Two possible explanations for the loss of antibiotic response were suggested. It was thought that there might have been a change in the microbial population of the animal room at about the time the change was noted, since the room was not cleaned with a hose as frequently as previously in order to decrease the humidity. (One could reason that this also might affect the results in the opposite manner since a clean environment is known to decrease response to antibiotics.) The other suggested explanation for the results was that there could have been a decrease in the number or virulence of the harmful microorganisms with the continued use of antibiotics. This suggestion is not substantiated by the weights of the various control chickens not fed antibiotics, which averaged the same during the first ten months as in the last fourteen months (211 and 213 g. respectively).

Another explanation, not considered by the investigators, is that under the continued use of the antibiotics there conceivably could have been an increase in the number of harmful microorganisms due to development of resistance of such strains to antibiotics. In this connection it is also pos-

sible that the antibiotic feeding during the first twenty-two months in some manner stimulated the growth of beneficial microorganisms which eventually did not respond to antibiotic feeding. The weights of the birds fed antibiotics tended to be higher during the first part of the experimental period than toward the end, providing some evidence for this possibility. Other possibilities exist. The above experiments do not permit any final explanation for the change in antibiotic effect.

It is unfortunate that each experiment covered only a three-week growing period. It is possible that the antibiotics might have shown a delayed growth-promoting response if the experiments had been continued for longer periods.

In spite of the weaknesses in the study, it is important since it indicates that the birds raised in an "old" environment eventually showed no extra growth stimulation when penicillin was added to the diet. Although similar results have not been reported under field conditions, the results should alert others to examine their growth records for similar effects. However, it is highly imperative that this type of study be carried out under more rigorously controlled conditions before it can be concluded that antibiotics will eventually lose their effectiveness as growth-promoting agents in animal diets.

INCREASED FOOD PRODUCTION BY GRASSLAND IMPROVEMENT

The extent of progress in the last two decades in a variety of problems concerned with farm production of foods is often overlooked. One phase of this concerns the improvement of pastures and the increase in hay production. Some interesting examples of the tremendous potential of our grasslands and of the demonstrated improvements that have been made possible in recent years have been given in a recent series of articles.

In the discussion of the grasslands of the cool, humid region of the United States, which includes all of New England, the Mid-Atlantic and North Central States, extending westward to include Minnesota, Iowa and Missouri, H. B. Sprague (*J. Agr. Food Chem.* **3,** *26* (*1955*)) points out that the estimated feed value of $25 per acre of pasture is undoubtedly too low, even for the present neglected status of grazing lands. However, this value indicates the great importance of this resource, in view of the fact that hay and pasture acreage combined account for 56.6 per cent of the total useful land area in this twenty-two-state area. One example of the importance of pasture lands in human food production is shown in the calculation that in 1938 New York pastures provided one third of the nutrients required yearly by dairy herds at only one seventh of the total feed bill cost.

Numerous experiments are described in which the amount of forage produced on permanent pastures without renovation can be improved by the use of top dressing with lime and fertilizer. In one of these experiments, increased forage production varying from 59 to 139 per cent was recorded. Further increases were possible when the pastures were renovated appropriately with the increases varying between 90 and 174 per cent. In other cases, thorough renovation of permanent pastures increased forage production by as much as 200 to 500 per cent for a period of three to five years after renovation, in contrast to the lower improvement noted when only top dressing was applied.

In a companion discussion concerning the warmer humid region of the United States, including the eastern one fourth of Texas and Oklahoma and the nine states directly east of them, G. W. Burton (*J. Agr. Food Chem.* **3,** *23* (*1955*)) comments upon some of the improvements that have been experi-

mentally demonstrated in pasture lands in this area. In the census of cattle and pasture lands conducted in 1949, it appeared that an average of 7.8 acres was required to supply the roughage for one animal in this region. This is appreciably below the 9.4 acres per animal unit required the nation over. He points out that this could be greatly reduced by clearing operations on certain of the pasture lands, by the introduction of certain types of grasses from tropical countries that do well in the soils of this region under the relatively heavy rainfall, by fertilizers and by insecticides. Under certain circumstances, irrigation during drought periods was desirable and economically feasible.

In contrast to the average number of acres required to maintain animals for the entire area, Burton describes one experiment in which coastal Bermuda grass, a plant particularly suitable for this area of the country, had been grazed by beef steers. When 36 pounds of nitrogen fertilizer with an appropriate amount of phosphate and potash were used, this grass forage was able to carry 1.33 steers per acre for the grazing season, with the production of 278 pounds of beef per acre. When the nitrogen addition was increased to 200 pounds, the carrying capacity increased to 3.5 steers, and the production of 696 pounds of beef per acre per year. Another example of the use of coastal Bermuda grass was described for a two-year period, 1952 and 1953, on sandy loam soil given different amounts of fertilizer containing nitrogen, phosphorus pentoxide and potassium oxide in a 4:1:2 ratio. When the hay was cut five times per year, 1.8, 5.3, 7.1, 9.7 and 10.6 tons of hay per acre were produced with 0, 100, 200, 400 and 800 pounds of nitrogen added per acre respectively. In addition to the increased production, the protein percentage of the hay on a dry basis increased from 7.1, 10.6, 11.5, 13.8 to 16.0. Total production cost based on current prices amounted to less than $10 per ton of hay when 200 or less pounds of nitrogen per acre were applied. The per ton cost rose to $17.79 when 800 pounds of nitrogen were applied per acre. On the dry weight basis, the higher levels of fertilizer were uneconomical. However, on a protein production basis, the costs were highly comparable.

An example of the use of insecticides was given for the summer of 1954 when forage was unusually short as a result of drought. One timely application of toxaphene, at a per acre cost of $3.25, saved from Army worms a two-ton cutting of coastal Bermuda hay, worth over $50 per acre.

The third of this series of articles considered the Great Plains region, constituting the vast heartland of the North American continent, stretching from the Great Plains in central Texas to the Canadian border and comprising some 630,000 square miles that originally had a dominantly rich grassland flora (J. R. Harlan, *J. Agr. Food Chem.* **3,** *29 (1955)*). One of the keen problems in this area now concerns weed control. In recent years there has been increasing inroad upon desirable pasture lands of a variety of heavy brush plants that dominate grasslands to the point of completely excluding normal grass growth in some areas. An example on weed control is of particular interest. The approximate cost of one treatment of a weed killer by airplane was $2.50 per acre. This single treatment controlled about 75 per cent of the brush. Over a ten-year period, this tract of land had an increased ability to carry stock of approximately 48 per cent. The annual net return from the brush control of pastures was $8.19 per acre, a gain of $2.57 per acre over areas where the pastures had not been treated. In other words, the cost of weed control was paid for by the increased profits of the first year, and the next nine years profits could be considered bonuses as a result of the weed killing. In other studies conducted over a six-year period, the net advantages were found to be even greater, with the stock-carrying capacity increased by 70 per cent and beef pro-

duction by 70 per cent, and with profit per acre of $3.18 in excess of untreated areas.

Harlan points out clearly that abandoned fields do not revert to grassland quickly and that the true climax may not be restored even after forty years of lying idle. Hence it is very desirable practice to seed these acres back to grass in order that they can be quickly used in conjunction with adjacent native range plans. It has been estimated that there are over 12,000,000 acres of abandoned fields in the Great Plains portions of Texas, Oklahoma, Kansas and New Mexico. The potential available for renovation of these areas is obvious.

In the editorial introduction to this series of articles, the prediction is made that fertilizers can boost pasture production by 50 to 100 per cent, supplemental irrigation by another 25 to 75 per cent, and pest control, winter crops, rotational grazing and other good management practices can add an additional 25 to 75 per cent more to forage production. While these procedures may not be strictly additive in their benefits, the over-all outlook is a very encouraging one that gives promise of abundant increases in food production as a result of increased pasture land. Two of the most hopeful facets are that quality, as well as quantity, is being improved, and the improvements can be made readily without increased ultimate cost to the consumer.

COTTONSEED PRODUCTS AS FOODSTUFFS

The quality of the proteins in cottonseed meal has been frequently discussed (*Nutrition Reviews* **1,** *178* (*1943*); **9,** *284* (*1951*); **12,** *171* (*1954*)). The toxic properties of cottonseed meal have been described and related to the amount of free gossypol in the meal (*Ibid.* **2,** *97* (*1944*); **3,** *167* (*1945*); **9,** *284* (*1951*)). Because of the economic importance of cottonseed meal to the cotton industry as a by-product and also to the livestock industry as a protein supplement, there continues to be intensive investigation of the best procedures to prepare the cottonseed meal for commercial use.

C. A. Cabell and I. P. Earle (*J. Agr. Food Chem.* **2,** *787* (*1954*)) have reported a series of collaborative studies in which the rat repletion method was compared with three other methods for assaying the nutritive values of the proteins in cottonseed meals. Seven laboratories took part in this comparison. In the first laboratory, the rat repletion method was used solely; in the second and third laboratories, the rat growth procedure was used; in the fourth, fifth and sixth laboratories, the chick method was employed, and in the seventh laboratory, a microbiologic procedure was tested. The protein quality of 12 samples of cottonseed meal prepared under a variety of circumstances was evaluated. No laboratory tested less than 8 samples; laboratories one and four tested all 12. A meal designated in the paper as S-676 was used as the standard for comparison with the other meals. This was a butanone-extracted product which was expected to show nearly maximum protein quality for cottonseed meal because it had been subjected to a minimum amount of heat, contained a low quantity of free gossypol, had a high nitrogen solubility, and a high thiamine content. In all laboratories this meal sample was given a value of 100 for its protein quality and the other samples were ranked proportionately. Three of the other cottonseed meals were made by the usual processing method in the plants producing these meals. The remaining cottonseed meals were ones that were prepared under specially designed circumstances of temperature and cooking in order to test the effect that these specific variations in procedure had upon the protein quality of the meal. Crude casein, crude casein plus 0.31 per cent tryptophan and 0.84 per cent methionine, defatted whole egg, and soybean

meal were used as other protein sources for comparison purposes.

In the rat repletion method, which was a modification of the procedure described by P. R. Cannon, E. M. Humphreys, R. W. Wissler, and L. E. Frazier (*J. Clin. Invest.* **23,** *601 (1944)*), the adult male rats were prepared for the test by feeding a low protein diet containing 0.1 per cent iodinated casein as the only source of protein for three weeks. The depleted rats were divided into 8 groups of 7 each according to body weight. They were then randomly assigned to the 8 protein-containing test feeds or supplements to be tested at any one time. The repletion period was for ten days, during which time the rats were fed the same basal diet except that the iodinated casein was omitted. In addition, the rats were fed the protein supplements in measured daily allowances sufficient to supply 0.1564 g. of nitrogen. The supplements were fed at the same time each day and were noted to be consumed completely and immediately. The meals were assayed twice in different trials with the same rats being used each time, but redepleted between trials. After each repletion the colony diet was fed for three days; the depletion diet was given for another seven days prior to another repletion trial. If the cottonseed meals were arranged in decreasing order of response in the rat repletion tests, they fell as follows: the butanone-extracted cottonseed meal had an equivalent protein quality to a standard soybean meal that was used as a control; low temperature screw-press meals and screw-press meals previously treated by hydraulic cooking; screw-press meals prepared by the customary procedure in the plants; prepress solvent-extracted meal; and finally, the lowest, a special experimental meal prepared by rupture of the pigment glands prior to cooking. The low rating of the last product was rather unexpected because it had a low free gossypol content and a fairly high nitrogen solubility.

The rat repletion method was positively correlated with all other methods except the procedure of chick growth in one of the three laboratories where chicks were used for assay, but was significantly correlated only with the microbiologic method. The most significant correlation of the methods used appeared to be among the three laboratories using the chick growth procedure. There was a uniform negative correlation for all the methods with the free gossypol content. Although statistically not significant for any one method, this was of considerable interest because the free gossypol content in these meals had been thought to be innocuous by reason of being less than 0.05 per cent. There was a negative correlation of all methods with cooking temperature, so that the meals prepared with the lower temperature had appreciably better protein quality. There was likewise a high positive correlation of most of the methods with the solubility of nitrogen in dilute sodium chloride solution, indicating that this value is of appreciable significance in measuring protein quality.

M. Z. Condon, E. A. Jensen, A. B. Watts, and C. W. Pope (*J. Agr. Food Chem.* **2,** *822 (1954)*) have studied the effect of autoclaving solvent-extracted cottonseed meal in the presence and absence of gossypol. The chick growth test was used for the evaluation of these effects. The investigators felt that this was a more sensitive test in view of the fact that low levels of gossypol influenced the growth of chicks to a greater extent than other test animals. The meal used in this series of experiments was produced from prime quality cottonseed by successive extractions with hexane and butanone to reduce the free gossypol content to 0.02 per cent and the total content to 0.2 per cent. Solvents were evaporated by a minimum amount of heat to avoid damage to the proteinaceous components. This type of meal has been well characterized because it has been used as a standard in nutritional investigations of cottonseed meals under a variety of conditions. The meal samples were spread in a layer 1.5 to 2.0 cm. thick in borosilicate glass dishes and autoclaved at

120°C. Prior to autoclaving, the meal sample was ground sufficiently to pass through a 2 mm. mesh screen. In some experiments, gossypol was added to the meals in a solution of acetone and evaporated at room temperature. In other experiments, gossypol was dissolved in prime quality refined and bleached cottonseed oil, and then mixed with the meal. Following autoclaving all samples were ground to pass a 2 mm. mesh screen. Meals to which oil had been added were extracted with commercial petroleum ether prior to grinding and re-extracted following grinding until the residual oil content was reduced to a level in the original meal of approximately 0.3 per cent. The residual free gossypol was removed from the autoclaved meals by extraction with 2 per cent aqueous butanone until the free gossypol content was 0.03 per cent or less.

The protein quality indices of the meals were determined by feeding trials in groups of 14 day old chicks. Except for minor modifications, the method used for the evaluation of protein quality was that of V. Heiman, J. S. Carver, and J. W. Cook (*Poultry Sci.* **18,** *464 (1939)*). After hatching, the chicks were placed for a ten-day period on a standardization ration composed of yellow corn meal fortified with vitamins and minerals. At the end of this time, the chicks were weighed and divided into groups by weight. The test ration was formulated to supply 12 per cent protein, 6 per cent supplied by yellow corn meal and the other 6 per cent supplied by one or another of the cottonseed meals under investigation. Vitamins and minerals were added in adequate amounts and equal amounts of starch and sugar were used to make the ration up to 100 parts. There was a slight increase in the protein quality of the cottonseed meal with five minutes of autoclaving, but thereafter a progressive decrease was noted as the length of autoclaving was increased. The nitrogen solubility of the meals in dilute sodium hydroxide was also decreased with increasing periods of autoclaving. There was no significant change in the soluble carbohydrate content after autoclaving for periods up to one hour. After autoclaving for two hours there was an indication of carbohydrate deterioration. After autoclaving for five minutes in the presence of 1 per cent gossypol mixed with the sample either in oil or in acetone, the total gossypol content of the meals was 0.9 and 1.0 per cent respectively. However, since the free gossypol content of all the meals was less than 0.03 per cent, the gossypol was bound almost quantitatively by other components of the meal. The chemical and physical properties and protein quality indices of the various cottonseed samples with gossypol appeared to be equivalent to those of the control samples autoclaved for a similar time without added gossypol.

Even though well prepared cottonseed meal is recognized as a good source of amino acids, its use is not recommended at present in the rations of laying hens. Cottonseed meals ordinarily contain sufficient gossypol to cause a marked discoloration of the egg yolks, especially when they are kept in cold storage. The yolks of such eggs may become reddish or olive-colored and show considerable mottling. The yolk membrane appears to be thickened and the yolk material is often gelatinous. C. R. Grau and co-workers (*J. Agr. Food Chem.* **2,** *982 (1954)*) have studied the nature of the component of the yolks of fresh eggs of hens fed gossypol. For most of these studies laying hens in the control series were fed a normal 16 per cent protein stock diet. The experimental hens were fed the same diet with an added source of gossypol. In most cases, gossypol was furnished in 1 per cent of hexane-extracted raw cottonseed meals or by 20 per cent of a commercially prepared, screw-pressed cottonseed meal. The first diet contained 0.0075 per cent of free gossypol and 0.095 per cent total gossypol. The second diet contained slightly more of each. Isolated gossypol was fed as a supplement in one experiment. The gossypol of uncooked meals is present in readily available form. During the cooking and screw-pressing operations in commercial

manufacture, most of the gossypol reacts with meal fractions to produce an undigestible bound form.

Many attempts were made to fractionate the egg yolks in order to differentiate normal eggs from eggs laid by hens fed gossypol. It was found that yolks of eggs from hens fed gossypol contained a yellow component that was not extractable by acetone but was soluble in a mixture of three parts of hexane to one of acetone. This extract contained a material that was related in amount directly to the level of gossypol fed in the diet. However, its absorption spectrum was different from that of gossypol. As yet the distinctive component of the egg yolk from gossypol-fed hens has not been identified chemically. Studies on discolored eggs taken from storage show that the distinctive parts of the absorption curve can still be detected after eighteen months. Thus the discoloration is not due just to a breakdown of this unusual yellow compound in the yolk during storage, but must involve some other component of the yolk which interacts in some way with the compound resulting from gossypol ingestion.

M. F. Stansbury, A. F. Cucullu, and G. T. Den Hartog (*J. Agr. Food Chem.* **2,** *692 (1954)*) have made a series of studies on the influence of the variety of cotton and the climatic and soil environment on the oil content of cottonseed kernels. These authors point out that, next to lint in economic importance, cottonseed oil is of the greatest interest and value to people in the cotton industry. They studied the oil content of moisture-free cottonseed kernels from 8 varieties of cotton grown in 13 locations during three years. The mean value varied from 35.4 to 37.7 per cent among the several varieties with an over-all average of 36.4 per cent. Within any one variety there was a much wider variation dependent upon the environment. For example, in the variety with the lowest avarage oil content, there was a range from 27.6 to 41.2 per cent. In the variety with the highest mean, a range was noted from 32.4 to 42.7 per cent.

The various environmental factors at different locations were found to cause appreciably greater variations than the actual variety influence. For example, the mean oil content over three years for the same 8 seed varieties at Statesville, North Carolina, was 34.1 per cent. This value was particularly low by reason of the fact that the middle year, 1948, had a 29.9 per cent oil value. In contrast, the 8 varieties over the same three years at St. Joseph, Louisiana, yielded a mean oil content in the moisture-free kernels of 40.0 per cent. Rainfall during seed maturation was found to have a significant influence on oil content when temperature was constant: the more rainfall the higher the oil content. Temperature during seed maturation significantly influenced oil content when rainfall was constant: the higher the mean maximum temperature the lower the oil content. The maximum temperature and rainfall appeared to be influences of nearly equal importance upon the oil content under the conditions of these studies. It was interesting to note that there was no statistically significant difference in the way the 8 varieties reacted to change in rainfall and temperature during seed maturation. Other environmental circumstances than those evaluated in these studies were believed to be important in the determination of the amount of oil in the cottonseeds.

The various aspects which are covered in the above studies indicate both the concern in the agricultural area for full knowledge about the commercial products of the cottonseed and also the intensive efforts which are being made to find satisfactory answers to the problems involved. One of the most interesting demonstrations from the above studies was the toxicity of gossypol at levels well below the 0.05 per cent level believed to represent the threshold. The need for an adequate market for cottonseed products and the continually increasing demand for inexpensive concentrates in the animal industry are forces which will continue to keep interest high in these phases of investigation.

DENTAL CARIES

While much experimental work is being done on the relation of diet to the incidence of dental caries, little attention has been paid to the possible effects of the physical consistency of the diet. In experiments designed to study the effect of increased mastication on the incidence of caries J. H. Shaw (*J. Nutrition* **54,** *177 (1954)*) has discovered interesting if perplexing facts.

In one experiment cotton rats of a caries-susceptible strain were weaned at 14 days of age and fed a caries-producing diet for fourteen weeks. The animals were then killed and the number and severity of the carious lesions were determined. While some of the cotton rats were maintained exclusively on the caries-producing diet, others received supplements of 10 per cent agar or cellu flour at levels of 10, 35, 50, 75, or 100 per cent. The object was to dilute the ration with a non-nutrient material to force a greater food intake and thus more extensive mastication. The addition of 10 per cent agar or of cellu flour at levels up to 50 per cent did not affect caloric intake or growth, the animals responding to the decreased caloric density of the diets with an appropriate increase in the quantity of food consumed. Only when the cotton rats received supplements of 75 and 100 per cent did they fail to grow as well as the controls receiving only the basal ration. The author had clearly succeeded in his objective to make the animals chew more extensively. However, the number of carious molars, the total number of lesions and the extent of the lesions were virtually identical for all groups of cotton rats tested. Only the 100 per cent cellu flour supplement caused an increase of doubtful significance in the number of molars involved and in the extent of the lesions.

In a second experiment, similar in design to the first, white rats of a caries-susceptible strain were employed. The cariogenic diet was supplemented for some animals with cellu flour at levels of 10, 35 and 50 per cent. Again the animals responded to the caloric dilution by increasing their food intake. No differences in the number and extent of tooth lesions were observed.

The third experiment involved the use of the synthetic resin polyvinyl acetate as a binder for the ingredients of the ration. Molten polyvinyl acetate (molecular weight 2400) was mixed with the ration and the preparation allowed to cool to form a hard cake about 1 inch thick and requiring considerable mastication. The polyvinyl acetate constituted a 20 per cent supplement to the basal ration. Another group of rats received the basal diet plus a 20 per cent supplement of finely powdered polyvinyl acetate. A third group of animals received the basal ration without further supplementation. The form in which the polyvinyl acetate was fed proved to be unimportant. By either method it caused a highly significant lowering of the number of carious molars, the total number of lesions as well as the severity of the lesions.

When powdered polyvinyl acetate was incorporated into the ration at 5, 10 and 20 per cent levels (experiment 4) there resulted a progressive lowering of the number and severity of the tooth lesions. The synthetic resin had no effect on the rate of growth of the experimental animals.

In the last experiment the effectiveness of the low molecular weight polyvinyl acetate used in experiments 3 and 4 was compared with that of a high molecular weight polyvinyl acetate, natural chicle and arochem. All supplements were fed at the 10 per cent level, and all were effective in reducing the incidence and severity of caries.

The author carefully points to the difficulties of explaining his findings. He concludes tentatively that a mere increase in mastication cannot explain the results, since the effort required to ingest diets supplemented with agar, cellu flour, powdered polyvinyl acetate, chicle and arochem should be nearly identical. Yet agar and cellu flour

were ineffective in preventing carious lesions. On the other hand the preparation most difficult to masticate, the diet cake prepared with molten polyvinyl acetate, was as effective but no more effective than the same ration in finely powdered form. The author entertains the notion that the effective supplements used in his experiments exerted some cleansing action in the oral cavity or specifically on the tooth surface. Another possibility might be that these substances deposited a protective film on the teeth. He noted that rats receiving the protective supplements had cleaner mouths and glossier teeth than did animals receiving the basal ration alone. Shaw points out that although polyvinyl acetate and chicle are common components of chewing gum, the results of these experiments should not be applied to human beings. There should be considerable difference between the effect of long-continued exposure to substances incorporated into the ration and the intermittent use of chewing gum.

W. Wynn, J. Haldi, J. H. Shaw, and R. F. Sognnaes (*J. Nutrition* **50,** *267 (1953)*) reported a striking difference in the cariogenicity of diets used at Emory and Harvard Universities. Both diets were of the purified type; both contained 64 per cent sucrose. The Emory diet contained 20 per cent casein, the Harvard diet 23 per cent. There were other small differences between the two diets with respect to fat, minerals and vitamins. The salt mixture employed in the Emory diet contained aluminum while that in the Harvard diet did not. Wynn and Haldi (*Ibid.* **54,** *285 (1954)*) investigated the possibility that aluminum might exert a protective action when added to a caries-producing diet. The basal ration consisted of 64 per cent sucrose, 20 per cent casein, 8 per cent fat, 4 per cent yeast and liver extract and 4 per cent salts. It did not contain added aluminum. Other diets were prepared from this basal one by adding aluminum at levels of 0.16, 2 or 20 parts per million. The diets were fed over a period of one hundred and eighty days. In one experiment the animals' salivary glands were removed after one hundred and twenty days on the diet. This procedure increased the number and severity of the carious lesions.

The incidence of caries and the severity of the tooth lesions were virtually identical in rats receiving the basal ration and in those receiving the aluminum-containing diets. As expected, removal of the salivary glands aggravated the condition of the molars, but the dietary aluminum level was without effect even in these animals. Growth, food intake and general physical condition were not influenced by the level of dietary aluminum. The difference in cariogenicity of the Emory and the Harvard diets cannot be attributed to the aluminum content of the former.

PANTOTHENIC ACID

Although the function of pantothenic acid as a constituent of coenzyme A is rather well understood, its role in pregnancy, and the comparative effectiveness of its conjugates in animal nutrition, are worthy of consideration.

A study of the role of pantothenic acid in the rat during pregnancy has been undertaken by G. Everson *et al.* (*J. Nutrition* **53,** *341 (1954)*). Twenty-eight day old rats from an inbred stock were raised to maturity and mated. Those females producing first litters of normal size, weight and appearance were selected for the experiment. These selected animals were divided into four groups. Group I continued to receive the stock ration on which the animals had been reared. Group II was fed a synthetic ration consisting of 25 per cent casein, 55 per cent dextrose, 15 per cent fat, and 5 per cent

salts. The diet was fortified with vitamins except for pantothenic acid and ascorbic acid. Group III received the same synthetic diet as Group II but was given in addition 100 micrograms of calcium pantothenate per day. Group IV was supplied with 1 mg. of calcium pantothenate per day in addition to the basal synthetic ration. The females in these four groups were mated a second time. The resulting offspring were removed immediately after parturition, before the young had an opportunity to nurse. The dams were killed and the pantothenic acid content of the liver and carcass was determined by microbiologic methods. The blood and carcass of the newborn rats were analyzed for pantothenic acid. Analyses were also made for ascorbic acid and for alkaline phosphatase of the serum of the offspring.

The breeding females transferred to the pantothenic acid-free ration (Group II) showed markedly reduced reproductive performance. Litter size was reduced to an average of 4.2 as compared with 11.2 for the control animals of Group I. The addition of 100 micrograms or 1 mg. of pantothenate per day raised litter size to 7.8 and 8.0 respectively (Groups III and IV). Average birth weights were 5.2 g. in Group I and 5.1 g. in Group IV. Lack of pantothenic acid in diets of Group II animals reduced the birth weights to 3.7 g. The addition of 100 micrograms of pantothenate per day (Group III) raised the birth weights to 4.7 g. The absence of dietary pantothenate frequently delayed parturition; in some instances the young were not carried to term. Occasionally resorption of the fetuses occurred even in animals receiving 1 mg. of pantothenate daily. The authors state that the reproductive performance of females even on the well-supplemented synthetic ration did not equal that of the control animals on the stock diet.

Maternal pantothenic acid levels in liver and carcass reflected the level of intake of this vitamin. Feeding the pantothenate-free diet resulted in the greatest reduction in the maternal tissue levels. Higher levels were observed when 100 micrograms of the vitamin were supplied daily. The stock ration, furnishing 450 to 600 micrograms of pantothenate per day, resulted in still higher levels. The daily administration of 1 mg. of pantothenate still further raised the tissue levels of this member of the vitamin B-complex. The pantothenate content of the blood and carcass of the newborn also reflected the maternal intake of the vitamin. The condition of the young did not always reflect faithfully the tissue pantothenate levels of mother or offspring.

Although the alkaline phosphatase and ascorbic acid levels of the blood of the newborn rats were virtually identical for the four groups studied, blood pyruvic acid levels were considerably elevated in Group II (no pantothenate) as compared with the stock diet group. Such an elevation of blood pyruvate levels suggests interference with the normal oxidation of carbohydrate similar to that of thiamine deficiency. This might be expected since pantothenic acid is required for the synthesis of coenzyme A. However, offspring of mothers receiving the high level of pantothenate (1 mg. per day) in the synthetic ration also showed some elevation of the blood pyruvate level. All in all the paper makes a valuable contribution to our knowledge of nutritional requirements in pregnancy. It also calls emphatic attention to the fact that complete synthetic diets are not nutritionally equivalent to complete stock rations.

A comparison of the nutritional effectiveness of pantethine and calcium pantothenate for the chick has been made by R. Q. Thompson, O. D. Bird, and F. E. Peterson (*J. Nutrition* **53**, *365 (1954)*). Pantethine is a conjugate of pantothenic acid and beta-mercaptoethylamine. It appears to be identical with the *Lactobacillus bulgaricus* factor (LBF) and constitutes a portion of the coenzyme A molecule. It may be assumed that pantethine is an intermediate in the biologic

synthesis of coenzyme A and acts as a special growth factor for the microorganism *L. bulgaricus* because the latter cannot rapidly combine pantothenic acid and mercaptoethylamine. Thompson *et al.* found that the chick responded equally well to pantethine and to pantothenic acid under most of the conditions employed. The chick apparently has no difficulty synthesizing pantethine (and through it coenzyme A), providing adequate dietary pantothenic acid is furnished as starting material.

MICROBIOLOGIC ACTIVITY OF THE METHYLSULFONIUM SALTS OF METHIONINE

Methionine is a key amino acid from a functional standpoint. In addition to serving as a structural unit of proteins, methionine is intimately involved in the transfer of methyl groups in biologic systems. The exact mechanisms involved and the relationships to other nutrients are as yet imperfectly understood.

It has been shown with *in vitro* technics that, in the systems employed, methionine is converted to an intermediate "active" form prior to transfer of its methyl group (*J. Biol. Chem.* **204,** *403 (1953)*; *Nutrition Reviews* **12,** *351 (1954)*). "Active methionine" has been shown by G. L. Cantoni (*loc. cit.*) to be S-adenosylmethionine, which is a sulfonium compound (*i.e.*, the sulfur is quadrivalent). These findings lend new interest to the biologic activity of another naturally occurring sulfonium compound, the methylsulfonium derivative of methionine, which was isolated from cabbage by R. A. McRorie *et al.* (*J. Am. Chem. Soc.* **76,** *115 (1954)*). The activity of this compound for growth of several strains and mutants of *Escherichia coli*, *Neurospora crassa*, and *Lactobacilli*, as well as the effect on reversal of sulfonamide inhibition of the *E. coli* organisms, have been reported by McRorie, M. R. Glazener, C. G. Skinner, and W. Shive (*J. Biol. Chem.* **211,** *489 (1954)*).

These workers found that methionine methylsulfonium iodide (MMI) was three to seven times as active as methionine for supporting growth and in reversing sulfonamide inhibition of a wild strain of *E. coli* (Texas) and two methionine-requiring mutants derived from it. For one of these mutants, MMI was more active than methionine in promoting growth than in reversing sulfonamide inhibition. However, MMI was much less effective than methionine in supporting growth of *E. coli* mutant 113-3, which requires either methionine or vitamin B_{12}. *E. coli* W (ATCC 9637), which is the parent strain of mutant 113-3, failed to respond to MMI when inhibited by sulfonamide, although methionine was active under these conditions.

Methionine had a sparing effect on the quantity of MMI required by one of the methionine-requiring *E. coli* mutants for growth in the absence of sulfonamide. This effect was not found upon addition to the medium of a mixture of compounds that function in one-carbon metabolism (xanthine, serine, histidine, glycine, thymine, choline, dimethyl-beta-propiothetin, para-aminobenzoic acid, vitamin B_{12}, folacin and folinic acid). In the absence of methionine the response to MMI was unaffected by this mixture of compounds.

Ethionine, the ethyl analogue of methionine, has been shown by many workers to be toxic to several species under varying conditions. In general, the adverse effects of ethionine could be reversed or prevented by adequate methionine. The ethylsulfonium derivative of ethionine (EEI), an analogue of MMI, was toxic to *E. coli* Texas. The growth suppression caused by EEI (3000 micrograms per 10 ml. of medium) was reversed by relatively small

quantities of methionine (15 micrograms) in the noncompetitive manner of a product of an inhibited enzyme system. At very high concentrations of EEI (10,000 micrograms) methionine did not reverse the inhibition at any of the concentrations of methionine tested. On the other hand, MMI reversed the inhibition of EEI in a competitive manner.

Using a series of *Neurospora* mutants, these workers found that the activity of MMI ranged from 0 to 95 per cent that of methionine. Variation in response was also found among the *Lactobacilli* tested. MMI did not support even partial growth of *Streptococcus faecalis* R or *Leuconostoc mesenteroides* P-60, an organism used for the assay of methionine. The sulfonium compound supported maximal growth of *Lactobacillus arabinosus* and *Lactobacillus casei* in a manner equivalent to methionine. At suboptimal levels, however, the sulfonium compound was two to three times more active than methionine.

It is apparent from the data that some of these organisms can convert the sulfonium derivative to methionine, since methionine is an irreplaceable constituent of cellular protein. The most significant finding lies in those experiments where the activity of MMI was several times higher than that of methionine, thus suggesting a separate function for the sulfonium compound. It is important to determine the relative activity of S-adenosylmethionine under these conditions.

The results of these studies with microorganisms point to the nutritional importance of the sulfonium compounds for animals and man. In 1941 M. A. Bennett (*J. Biol. Chem.* **141,** *573*) reported that the methylsulfonium derivative of methionine could replace methionine for the growth of rats on a methionine-deficient diet. More recently, J. A. Stekol, S. Weiss, E. I. Anderson, and P. T. Hsu (*Abstr. 126th Meeting Am. Chem. Soc., 19C (1954)*) reported that a methylmethioninesulfonium salt containing C^{14} as the alpha and methyl carbons was not incorporated into tissue protein as readily as similarly labeled methionine. Contrary to the report of Bennett (*loc. cit.*), Stekol *et al.* found that their sulfonium compound did not support growth of rats to the same extent as methionine; however, its methyl groups were incorporated into choline or creatine as well as or better than the methyl group of methionine. It thus becomes of practical importance to learn more concerning the nutritional significance of naturally occurring sulfonium compounds, as well as their formation and function in the living organism.

VITAMINS AND AMINO ACID SYNTHESIS

The catalytic functions of the B-vitamins in cellular oxidation are widely appreciated. Similarly, the role of pyridoxine in deamination and transamination reactions has been extensively explored. Knowledge of the specific functions of vitamins in amino acid metabolism has been accumulated more slowly. Some of these functions are already known: folacin and vitamin B_{12} in the metabolism of methionine and choline, ascorbic acid and the oxidation of tyrosine, etc. The role of folacin in the interconversion of the amino acids glycine and serine has been investigated in detail by R. L. Blakley (*Biochem. J.* **58,** *448 (1954)*). The problem is of special interest, since the transformation of the two amino acids involves the addition or removal of a single carbon, analogous to the interconversion of homocysteine and methionine.

Preliminary experiments showed that incubation of glycine, labeled at the number two position with radioactive carbon, plus unlabeled serine gave rise to serine labeled

at the number two position. The conversion was catalyzed by extracts or homogenates of pigeon liver, as well as by liver powders obtained by acetone extraction, and required in addition a boiled yeast extract and folacin. These data suggested that the enzymes of liver together with folacin and the co-factors in yeast extract catalyzed the removal of the third carbon of serine and its addition to the labeled glycine, giving rise to labeled serine. The bulk of the paper was devoted to elucidating the mechanism of this transfer and the mode of action of the co-factors involved.

Incubation of dialyzed liver extract with labelled serine and folacin gave rise to detectable amounts of glycine and formaldehyde. Yeast extract was not essential for this cleavage. On the other hand, dialyzed liver extract and folacin were capable of converting glycine and formaldehyde into serine. Formic acid did not condense with glycine under these conditions. These observations suggest that glycine and serine are interconvertible by the removal or addition of formaldehyde. It is in these terms that the results of the preliminary experiment must be interpreted.

Further experiments demonstrated that the need for folacin and yeast extract was particularly critical when the enzyme preparation (liver extract) was dialyzed prior to use. Undialyzed liver extract was fully active in the absence of added folacin and of yeast extract. On the basis of previous reports from other laboratories vitamin B_{12}, pyridoxal phosphate and homocysteine might be expected to be co-factors in the glycine-serine interconversion. However, under the conditions employed by Blakley, addition of these compounds was ineffective, although a combination of adenosine triphosphate and diphosphopyridine nucleotide was capable of replacing the yeast extract. Adenosine triphosphate alone possessed considerable activity.

Leucovorin, previously implicated in the enzymatic transfer of one-carbon fragments, possessed the same activity as folacin in catalyzing the glycine-serine interchange. There was, however, no evidence that folacin was transformed into leucovorin before exerting its catalytic action. Tetrahydrofolacin and its N^{10}-formyl derivative were the only compounds found to be more active than folacin itself, particularly in the absence of the yeast extract. Comparison of tetrahydrofolacin with folacin plus yeast extract showed the former to possess much greater activity during the early hours of incubation while the latter increased markedly in strength after several hours. This suggested that the yeast extract might be involved in converting folacin to the active coenzyme while the tetrahydro derivative required little or no transformation. The author suggests a scheme in which tetrahydrofolacin plays the central role as the coenzyme accepting and then donating formaldehyde during the serine-glycine transformation. He suggests that folacin itself may be transformed into the tetrahydro derivative by yeast extract or by adenosine triphosphate plus diphosphopyridine nucleotide.

The synthesis of serine from glycine by *Streptococcus faecalis* R has been studied by J. Lascelles and D. D. Woods (*Biochem. J.* **58,** *486 (1954)*). Since these authors were particularly interested in the roles of folacin and pyridoxine, the work was done largely with cells made deficient in these two vitamins by growing them on a synthetic medium containing no pyridoxine and only a trace of folacin. The amount of serine synthesized under various conditions was determined by microbiologic assay.

Folacin- and pyridoxine-deficient cells synthesized significant quantities of serine only when incubated with glycine, formate, glucose, folacin and pyridoxal. Omission of any of these factors reduced serine synthesis to about 10 per cent of the value obtained with the complete medium. Formate was the only fully effective carbon source for the condensation with glycine.

The methyl groups of methionine, choline, betaine and sarcosine were not utilized. Formaldehyde was ineffective, in sharp contrast to the liver system used by Blakley (*loc. cit.*), in which formaldehyde condensed with glycine while formate did not. Carbon dioxide did not serve as a source of carbon in serine synthesis by this streptococcus. Certain other microorganisms are known to be capable of utilizing carbon dioxide for this purpose. The formyl derivative of folacin was as active as folacin itself. Leucovorin and formylpteroic acid with glutamate showed considerable activity but were somewhat less effective than folacin.

When cells were incubated with folacin before being tested for their ability to synthesize serine, the vitamin was apparently bound to some cell component. Subsequently they could convert glycine and formate into serine in the absence of added folacin. If the cells were first incubated with folacin plus formate, with leucovorin, or with the formyl derivative of folacin, subsequent serine synthesis was greater than when prior incubation was done with folacin alone. These observations, together with the finding that the analogue methylpteroic acid interfered with the function of folacin in serine synthesis but had much less effect on the action of leucovorin, suggested that folacin is transformed into leucovorin or some related substance and bound as a coenzyme.

Lascelles and Woods also found that the synthesis of serine was accompanied by the conversion of a part of the folacin present into a substance which supported the growth of *Leuconostoc citrovorum*. This material is probably closely related to leucovorin. Its synthesis occurred when folacin, formate and glucose were present in the medium. Glycine and pyridoxal were not required.

The evidence in this paper suggests that cells of *S. faecalis* R effect the combination of folacin and formate into a substance closely related to leucovorin. It may be that this substance makes the final transfer of the one carbon needed to convert glycine into serine.

The papers of Blakley and of Lascelles and Woods have in common the tentative conclusion that the glycine-serine interchange requires the mediation of a folacin derivative which is capable of transferring the formyl groups. There are sharp differences between the findings in the two papers. The liver preparation of Blakley did not require pyridoxine. The source of carbon was formaldehyde (formate was not utilized). The suspension of microorganisms used by Lascelles and Woods required pyridoxine and incorporated only formate.

NUTRITION AND PREGNANCY

Growth is a convenient, much used and reliable criterion of nutritional adequacy. It has its limitations since it determines dietary adequacy with respect to only one aspect of an animal's nutritional needs. The literature offers numerous examples showing that purified diets which support superior growth may be mediocre or inadequate in supporting reproduction and lactation. While the need for more than a single criterion of nutritional adequacy is widely recognized, not all nutritionists have the patience to carry out the tedious and time-consuming experiments involved in studying the nutritional requirements for reproduction and lactation, nor would the results of such studies be necessarily applicable to other conditions. Studies of this sort have, however, immediate practical significance in view of the vital importance of nutrition in human pregnancy, its bearing on maternal and child health with all its medical, social and economic aspects.

A number of recent papers have dealt

with interesting aspects of nutrition in relation to reproduction and lactation. H. J. Deuel, C. R. Martin, and R. B. Alfin-Slater (*J. Nutrition* **54,** *193 (1954)*) have presented results which extend suggestions in the early literature that fatty acids are required for successful pregnancy and lactation in the rat. To investigate the quantitative requirements for essential fatty acids they placed weanling female rats on a fat-free diet for a period of ten weeks. At this time the females were bred with normal males from the stock colony. At the same time supplements of varying amounts of cottonseed oil or methyl linoleate, a derivative of one of the essential fatty acids, were administered, except to the control group which was continued on the fat-free ration.

That fetal development is largely independent of the maternal fatty acid intake was indicated by the fact that of two groups of fat-deficient pregnant females 83 per cent and 87 per cent cast litters. However, litter size was smaller than for the groups receiving supplements, and none of the young survived the first three days of life.

The addition of 10 mg. of cottonseed oil to the daily ration improved the number of young per litter as well as their survival during the first three days of life. The average weight of the young at 3 and 21 days of age was less than that obtained with larger supplements of oil. Mortality between 3 and 21 days was high. A daily supplement of 100 mg. of cottonseed oil supported a high percentage of litters born and a high average number of young per litter. Survival and rate of growth during the twenty-one days of the lactation period were good. Supplementation of the diet with methyl linoleate in adequate amounts was almost as effective as the cottonseed oil supplement (the number of young per litter was appreciably less when methyl linoleate was used than when cottonseed oil was added). The authors conclude that optimum results are obtained with 200 mg. of cottonseed oil or 100 to 200 mg. of methyl linoleate per day.

A study of maternal nitrogen storage and other changes in maternal tissue composition has been made by G. H. Beaton, J. Beare, M. H. Ryu, and E. W. McHenry (*J. Nutrition* **54,** *291 (1954)*). All experimental and control animals were individually caged and were fed a commercial stock ration. In the nitrogen balance study urine and feces were collected daily but pooled for three-day periods throughout the gestation period as well as six days post partum. During only two of the eight three-day periods was there any significant difference in nitrogen retention between the pregnant rats and the nonpregnant controls.

Fetal growth, and with it maternal weight, was greatly increased after the fifteenth day of gestation. During these last six days of pregnancy the liver increased in weight, partly but not entirely due to increased water content. Maternal carcass fat increased up to the fifteenth day of gestation and then declined sharply. The decrease in fat was accompanied by a transient increase in carcass protein. Carcass water was elevated during the entire course of pregnancy.

Analysis of some blood constituents revealed a reduction in packed cell volume, in hemoglobin and, for a brief period, in blood amino nitrogen. *In vitro* studies with liver slices gave some evidence that the liver's ability to synthesize urea was decreased during the last six days of gestation. The site of the impairment in urea synthesis could not be identified by measurement of liver arginase or by the effects of ornithine and citrulline on urea synthesis. A marked decrease in the liver alanine-glutamic transaminase was observed after the fifteenth day of pregnancy. Since these effects on urea synthesis and transaminase are similar to those following growth hormone administration (G. H. Beaton, G. Ozawa, J. R. Beaton, and E. W. McHenry, *Proc.*

Soc. Exp. Biol. Med. **83,** *781 (1953)*) the authors suggest that the various sharp changes occurring at about the fifteenth day of gestation may be due to increased growth hormone secretion.

The relationship between ascorbic acid and pantothenic acid has received further attention by G. Everson, L. Northrop, N. Y. Chung, and R. Getty (*J. Nutrition* **54,** *305 (1954)*). Large doses of ascorbic acid have been shown to ameliorate the effects of pantothenic acid deficiency. Everson *et al.* have determined the effect of ascorbic acid on the reproductive performance of rats receiving a diet devoid of pantothenic acid. Female rats of proven fertility were fed (1) the basal purified ration containing neither ascorbic acid nor pantothenic acid, (2) the basal ration plus 100 micrograms of calcium pantothenate per day, or (3) the basal ration plus 2 per cent ascorbic acid. The females were then bred with normal males. The animals receiving the ration devoid of both pantothenic acid and ascorbic acid cast litters with fewer and smaller young than did the females receiving the pantothenate supplement. The offspring, analyzed at birth, showed less pantothenic acid in blood and carcass. Serum alkaline phosphatase and ascorbic acid levels of the young were not affected, while blood pyruvic acid increased. Livers and carcasses of the deficient mothers showed a lower pantothenic acid content than did females supplemented with pantothenic acid. The addition of ascorbic acid to the diet in place of pantothenic acid increased the birth weights of the young almost to the level of offspring from pantothenate-fed rats. Blood, liver and carcass pantothenate levels of mothers and young were increased as much as in the pantothenic acid group. The number of offspring per litter was not greatly increased by the ascorbic acid supplement.

MICROBIOLOGIC ASSAY OF VITAMIN B_{12} IN MILK

Milk is known to be a good source of vitamin B_{12} for infants, children and adults. For infants, in fact, milk is usually the sole dietary source of vitamin B_{12}. Because of this, studies on the assay of vitamin B_{12} content of milk are of basic importance. The vitamin B_{12} content of milk of various species has been previously discussed (*Nutrition Reviews* **10,** *219 (1952)*; S. K. Kon and K. M. Henry, *J. Dairy Res.* **21,** *245 (1954)*).

Methods of analyzing the milk of various species by microbiologic assay have been studied by M. E. Gregory (*Brit. J. Nutrition* **8,** *340 (1954)*). Three different organisms were used, *Lactobacillus leichmannii*, *Escherichia coli*, and the protozoan *Ochromonas malhamensis*. The last-named organism has been shown to be quite specific for forms of vitamin B_{12} with animal activity (J. E. Ford, *Ibid.* **7,** *299 (1953)*).

Milk was collected from cows, goats, sows, rats and ewes and compared with human milk. Various methods of preparing the samples of milk for assay were compared. When human and sow's milk were assayed, it was found that digestion with papain was necessary in order to release bound forms. In assaying the milk of other species, only variations of the procedures for determining vitamin B_{12} (dilution with water and addition of cyanide ions) were necessary for maximum assay values. However, ultrafiltration experiments indicated that vitamin B_{12} existed in the bound form in milk from all the species tested. Apparently, it is bound more firmly in human and sow's milk than in the other species. Alkali treatment of the milk destroyed all vitamin B_{12} activity, indicating the lack of vitamin B_{12}-sparing desoxyribosides.

When the milk samples were compared by assay with the three different organisms, the results were similar. This indicated that

the vitamin B_{12} activity of the milks was due mainly to cyanocobalamin, the form of this vitamin with animal activity. The average values for the various milks, in millimicrograms per milliliter, were as follows: cow, 2.3; goat, 0.9; human, 0.3; sow, 1.7; rat, 13.0; and ewe, 7.0. Values for colostrum of the cow and goat were 3.7 and 6.5 millimicrograms per milliliter respectively. It is of interest that unheated cow's milk, added to sterile assay tubes just before inoculation, depressed the growth of *L. leichmannii*, indicating the presence of an unknown inhibitor. Although only a few samples from each animal were used, the assay results compared reasonably well with other values in the literature cited by the author (*see also* Kon and Henry, *loc. cit.*).

The vitamin B_{12} requirement for children has not been established. Cow's milk is a good source of this vitamin since one pint provides approximately 1 microgram. This amount of vitamin B_{12} produces a good hematologic remission when injected daily in a patient with pernicious anemia. The much lower level of vitamin B_{12} in human milk might be the cause of some concern. However, in this regard it should be pointed out that breast-fed infants are not known to show any signs of vitamin B_{12} deficiency under normal conditions. This means either that the requirement of the infant for this vitamin is relatively low, or that its stores are adequate until such time as it secures supplemental sources of vitamin B_{12}. Also, it is possible that milk contains other nutrients which reduce the infant's requirement for this vitamin. Further studies are needed on the adequacy of vitamin B_{12} in the milk of human beings, cows, and other species, and on the possible vitamin B_{12} requirement of children.

THE EFFECT OF ALCOHOL ON THE CHOLINE REQUIREMENT

The relationship between the chronic ingestion of alcohol and the production of hepatic cirrhosis in man has been the subject of considerable controversy. There is no doubt that alcohol may displace the lipotropic factors from the diet and therefore contribute to liver necrosis. The possibility that alcohol might in addition have some "toxic" or direct detrimental effect has been suggested frequently without much substantiation. The experiments of C. H. Best, W. S. Hartroft, C. C. Lucas, and J. H. Ridout (*Brit. Med. J.* **2,** *1001* (*1949*)) are of importance in this regard. These investigators observed fatty infiltration and fibrosis of the livers of rats provided with a fluid intake of 15 per cent alcohol and a diet marginal in lipotropic activity. Rats receiving the same basal diet with sucrose substituted for the calories contributed by alcohol also incurred similar hepatic lesions. Rats receiving only the basal diet without either sucrose or alcohol supplementation did not develop lesions. These experiments were interpreted as showing that the harmful effect of alcohol could be attributed to its caloric contribution to the diet.

This experimental approach has been extended by G. Klatskin, W. A. Krehl, and H. O. Conn (*J. Exp. Med.* **100,** *605* (*1954*)) to show that alcohol increased the choline requirement of rats not solely by augmenting the caloric intake. As in the experiments reported above, rats given a fluid intake in the form of 15 per cent alcohol and a diet marginal in lipotropic activity developed fatty infiltration and fibrosis of the liver. Pair-fed animals which received an isocaloric amount of sucrose instead of alcohol also developed similar changes, but pair-fed rats receiving neither sucrose nor alcohol did not develop such lesions. Development

of hepatic lesions in rats given the basal diet ad libitum was attributed to the fact that such rats grew more rapidly, thereby increasing their choline requirement to a greater extent than the choline intake was increased. An effect of alcohol not attributable to caloric supplementation was shown by feeding a group of rats the same amount of alcohol but decreasing the sucrose of the diet by an isocaloric amount so that the total quantity of lipotropic materials and calories was the same as that given to the pair-fed controls not receiving either alcohol or sucrose. In contrast to the pair-fed controls, considerable fatty infiltration and fibrosis occurred in the experimental animals. The introduction of either choline or methionine into the basal diet was effective in preventing the fatty livers induced with supplements of either sucrose or alcohol.

A second paper by the same authors concerns the influence of alcohol in inducing renal necrosis in weanling rats fed a diet marginal in lipotropic substances (G. Klatskin and W. A. Krehl, *J. Exp. Med.* **100,** *615 (1954)*). The basal diet with a fluid intake of 15 per cent alcohol was provided ad libitum to the weanling rats in group A. Rats of group B received by pair-feeding the same amount of the basal diet with sucrose replacing the calories derived from alcohol in group A. Group C received the same amount of basal diet without alcohol or sucrose supplementation. The basal diet was fed ad libitum without alcohol to group D. The incidence and severity of the renal lesions in group A after one and two weeks was much higher than in the two pair-fed control groups B and C. However, on this diet the control group allowed to eat ad libitum (group D) showed the most severe lesions. The development of lesions in this group was again attributed to the increased weight gain and higher caloric intake.

Because it was concluded that alcohol acted by increasing the requirement for choline an attempt was made to quantitate this action. It was estimated that the basal diet contained the equivalent of 0.089 per cent choline. The effect of the addition of 0.08, 0.12 and 0.20 per cent choline to the basal diet on the development of renal necrosis in rats receiving alcohol or in either pair- or ad libitum-fed controls was observed. It was found that as little as 0.08 per cent choline was effective in preventing the renal lesions of choline deficiency.

The authors are to be congratulated on the care exercised in planning and carrying out these experiments. Under the highly restricted conditions of these studies it is apparent that the addition of alcohol to a diet deficient in lipotropic substances increases the incidence of fatty infiltration of the liver of adult rats and the renal lesions of choline deficiency in weanling rats. This effect is not dependent on an increase in caloric intake. Further information concerning the mechanism of action of alcohol is not yet available. The fact that supplementing the diet with only 0.08 per cent choline overcame the adverse effect of alcohol indicates that the augmentation of choline requirement is not of great magnitude. The authors suggest that their observations might apply in explaining the incidence of cirrhosis among alcoholics. They point out that the withdrawal of alcohol and the maintenance of a suboptimal dietary intake often leads to improvement in the function of the liver in patients with Laennec's cirrhosis. The incidence of Laennec's cirrhosis is higher in chronic alcoholics than in equally poorly nourished individuals with other diseases, and Laennec's cirrhosis is occasionally seen in heavy drinkers whose dietary intake has been reasonably well maintained. The problem of proving experimentally that alcohol increases the requirement of lipotropic substances in man will be very difficult.

THE ADRENAL CORTEX IN DIABETIC ACIDOSIS

The metabolic effects of insulin withdrawal include not only hyperglycemia, ketonemia and acidosis but also negative nitrogen and potassium balances. These latter two effects and some others less completely studied also occur after "stress," and after the administration of adrenocorticotropic hormone (ACTH) or cortisone. Taken together this suggests that increased adrenal hormone activity occurs in diabetic acidosis. Whether diabetic acidosis stimulates adrenal function by itself or as a result of nonspecific stress is the subject of investigations by J. W. McArthur *et al.* (*J. Clin. Invest.* **33,** *420, 437 (1954)*). It is well known also that hypophysectomy and adrenalectomy will reduce the severity of diabetes in animals and conversely that ACTH and cortical hormone administration (as for example in man in Cushing's disease) may produce insulin-resistant diabetes.

The experimental approach used by these workers was to study insulin withdrawal in dogs rendered diabetic by pancreatectomy. As measures of the effect of insulin withdrawal upon adrenal cortical function the authors counted the total neutrophils, lymphocytes and eosinophils in the blood and the urinary excretion of corticosteroids, the latter by the method of N. B. Talbot, A. H. Saltzman, R. L. Wixom, and J. K. Wolfe (*J. Biol. Chem.* **160,** *535 (1945)*). They first compared the time at which adrenal stimulation appeared with the occurrence of abnormalities in sodium, potassium, magnesium, phosphorus and nitrogen balances. Second, they conducted a similar study, but induced adrenal response by the administration of ACTH before the metabolic abnormalities previously observed and postulated to be due to adrenal hyperactivity would have occurred following a simple withdrawal of insulin. Third, insulin was withdrawn from an animal when both the pancreas and the adrenals had been removed. The studies were conducted with great care, using long control periods postoperatively before the experimental phase was begun, in this way increasing the ability to interpret results.

Evidences of adrenal cortical stimulation (by blood counts and hormone excretion) did not occur, as a rule, until thirty-six hours after insulin withdrawal. In contrast to human diabetic acidosis, negative sodium balance was much less marked or not observed at all. Potassium, phosphorus and nitrogen balances, however, were negative after insulin withdrawal, as they are in man. Negative nitrogen and phosphorus balances were moderate in degree until thirty-six hours after insulin withdrawal when they became much more marked. The negative potassium balance, initially much more marked than nitrogen balance (the excess potassium presumably coming from glycogen), returned nearly to equilibrium, and only became markedly negative again thirty-six hours following insulin withdrawal.

The sensitivity of one animal to insulin was determined using standard doses of insulin. Again, there was no change in insulin sensitivity during the first thirty-six hours after insulin withdrawal (three determinations at twelve, twenty-four, and thirty-six hours, respectively). At forty-eight hours and again at sixty hours there was a considerable decrease in sensitivity, again correlating with the period of eosinopenia and increased corticoid excretion.

It might be noted that all of the animals became much more sick thirty-six hours following insulin withdrawal; vomiting became severe and several of the animals died in spite of the institution of treatment.

When ACTH was administered intramuscularly (20 mg. every three hours for twelve hours) to a depancreatized dog twenty-four hours after insulin withdrawal (that is, twelve hours before adrenal stimulation would be expected), an acceleration of the acidosis and of the metabolic disturb-

ances appeared. Vomiting began early, and negative nitrogen balance increased strikingly with a concomitant increase in negative phosphorus balance. Potassium balance, which was negative out of proportion to nitrogen balance during the first twelve hours after insulin withdrawal as in the previous dogs, returned during the second twelve hours to a value approximately equivalent to the mild negative nitrogen balance. During ACTH administration in the third twelve-hour period, however, potassium balance again became strikingly negative and far out of proportion to the increased negativity of the nitrogen balance.

In another experiment both pancreatectomy and adrenalectomy were performed on the same dog, and after a suitable control period with the animal maintained on cortisone and desoxycorticosterone acetate throughout, insulin was withdrawn. This dog was thought to have a somewhat milder course than the animals with intact adrenals. In addition, although eosinopenia occurred, it was not so striking as in the previous studies and was not associated with polymorphonuclear leukocytosis as before. Potassium balance again became negative during the first twelve hours, but thereafter was either positive or in a state of equilibrium. The degree of negative nitrogen and phosphorus balance also was much less than in the nonadrenalectomized animals. It seems evident that body tissue was burned to a lesser degree in the doubly operated animal than in those depancreatized alone and that in the depancreatized animal given ACTH a stimulus to protein catabolism was evident.

The timing of the appearance of evidence of adrenocortical stimulation and its relation in time to the increased negative nitrogen, phosphorus and potassium balances, together with the mimicking of this reaction by ACTH when it was not expected, and the partial amelioration of the reaction by adrenalectomy, indicate that the latter part of the reaction of diabetic acidosis was due to adrenal cortical stimulation.

DIETARY FACTORS AND EXPERIMENTAL VASCULAR LESIONS

A relationship between cardiovascular lesions and various dietary factors is suggested by several recent investigations. Vascular lesions consisting primarily of aortic sclerosis and deposition of abnormal amounts of lipid in the aorta and coronary vessels have been observed in rats fed choline-deficient diets in both chronic and acute experiments (W. S. Hartroft, J. H. Ridout, E. A. Sellers, and C. H. Best, *Proc. Soc. Exp. Biol. Med.* **81,** *384 (1952)*; G. F. Wilgram, Hartroft, and Best, *Science* **119,** *842 (1954)*). Hypercholesterolemia has been produced in the Cebus monkey by feeding large amounts of cholesterol with a diet deficient in methionine, but adequate in choline. Supplements of methionine or cystine prevented and reversed the hypercholesterolemia. About half the monkeys demonstrating serum cholesterol levels above 300 mg. per cent for eighteen weeks or more developed marked atheromatous aortic lesions (G. V. Mann, S. B. Andrus, A. McNally, and F. J. Stare, *J. Exp. Med.* **98,** *195 (1953)*; *Nutrition Reviews* **12,** *25, 325 (1954)*).

The effects of choline on cardiovascular lesions which result from feeding large doses of vitamin D are reported by G. F. Buckley and W. S. Hartroft (*Am. J. Clin. Nutrition* **2,** *396 (1954)*). Male Wistar rats (weighing 120 to 150 g.) were used for these experiments which employed a basal choline-deficient, high fat diet. Twenty-five rats received the basal diet, 25 the basal diet supplemented with 0.85 per cent choline chloride, and 33 rats the diet supplemented with 15,000 to 20,000 I.U. of vitamin D

(calciferol in corn oil). Thirty-three additional animals received supplements of both choline and vitamin D. The animals were killed after thirty-one to forty-two days on the experimental diets, and the heart, aorta, liver, kidney, spleen and pancreas were examined histologically.

The well established consequences of choline deficiency on the liver and kidney tissues occurred. The livers of all animals maintained on choline-deficient diets contained abnormal quantities of stainable fat. Choline-deficient animals also demonstrated kidney damage, which was especially apparent in those rats also given the vitamin D supplement. These animals showed an increased amount of calcium deposition in the kidneys; otherwise the renal damage noted was similar to that reported previously and attributable to choline deficiency (W. S. Hartroft, *Brit. J. Exp. Path.* **29,** *483 (1948)*). Abnormalities did not occur in the livers and kidneys of the rats given choline whether or not vitamin D was added to the basal diet.

Aortic sclerosis occurred in 9 of the 25 rats fed the basal choline-deficient, high fat diet. Similar lesions were observed in 19 of the 33 animals fed the same diet supplemented with vitamin D. The addition of vitamin D to the choline-deficient diet thus resulted in an increased incidence of aortic sclerosis. Aortic sclerosis did not occur in 25 rats fed the basal diet supplemented with choline, nor in 33 rats given the same ration supplemented with choline and vitamin D. These findings indicate a protective effect of choline on the degenerative aortic lesions which develop in rats given large amounts of vitamin D and diets providing suboptimal amounts of choline. The incidence of coronary arterial sclerosis in the groups of animals studied was similar to that of aortic sclerosis. The protective effect of choline on arterial coronary sclerosis, however, was not so complete as it was for aortic lesions. Five of the 33 rats fed the basal ration supplemented with choline and vitamin D demonstrated coronary lesions, while none of these animals developed aortic lesions. An explanation for these differences is not apparent.

The mechanism involved in the production of vascular lesions in the experimental animal by choline deficiency or by the administration of vitamin D in large amounts in the presence of choline deficiency is not known. Two possibilities suggest themselves. First, the lesions may be a direct consequence of choline deficiency with resultant alterations in the metabolism of lipids by the blood vessels, just as it is thought that choline deficiency alters lipid metabolism in the liver and in the kidney. An alternate explanation is that the vascular lesions may result secondarily and indirectly from the renal lesions produced by choline deficiency. As Buckley and Hartroft point out, it is possible that the kidneys of the choline-deficient, vitamin D-supplemented animals did not excrete calcium effectively. Disturbances in calcium and phosphorus metabolism resulting from vitamin D administration in the presence of renal damage due to choline deficiency might then account for the experimental results. Measurements of calcium balance and of serum calcium and phosphorus were not done in these experiments. A definitive explanation for the mechanism of production of the vascular lesions in the studies cited above awaits further investigation.

It is difficult to translate the results of studies relating experimentally produced vascular lesions to lipotropic factors into the problems of vascular disease in human beings. There is no valid evidence that lipotropic factors are related to the development of atherosclerosis in man, or that these agents are of therapeutic value in the human being.

NOTES

Letter to the Editor

Dear Sir:

I was much interested in the letter of Dr. Pollack (*Nutrition Reviews* **13,** *31 (1955)*). During my stay in Thailand, I became interested in the nutrition of these people and was associated with Dr. Amara Chandrapanond of the Ministry of Public Health in this work. Dr. Amara Chandrapanond and her associates made dietary surveys in the northeastern and southern parts of Thailand, and also in the city of Bangkok. In all of these areas, their calculations indicated that these people were getting less than 300 mg. of calcium daily. At the time, I was struck by this low calcium intake, but apparently there did not seem to be any evidence of calcium deficiency.

The rice millers do not add any "stone powder" during the milling process. Moreover, if there were any calcium carbonate added, I am sure that it would be washed off during the washing process of the rice or lost during the cooking process. The Thais, in preparing their rice, wash it thoroughly before cooking it, and after cooking discard the water used in the cooking.

Most of the calcium in Thai diets is derived from dried, salted, or fermented fish. In the northeastern part of Thailand, Dr. Amara informs me, the people consume an average of 16 to 20 g. of fermented fish per day, and analyses by the Department of Medical Sciences reveal that fermented fish contains 1126 mg. per cent of calcium.

In much of Thailand and the neighboring countries of Southeast Asia calcium intakes calculate to less than 300 mg. daily. To my knowledge there is little or no evidence that health is impaired at such intakes, and there is recent evidence that calcium balance in adults is readily maintained at such levels (D. M. Hegsted, I. Moscoso, and C. Collazos, *J. Nutrition* **46,** *181 (1952)*). It is entirely possible that the generous calcium recommendations of American nutritionists (0.8 to 2.0 g. daily) represent artificial and unrealistic levels.

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Tributes to Scientists

Friends of Professor Leonard A. Maynard and Professor James B. Sumner arranged a special program of scientific papers and a dinner meeting to honor them before their retirement at the end of the present academic term. The meeting was held on the campus of Cornell University, Ithaca, New York, May 25 and 26.

The University of Illinois has arranged a reception, dinner meeting and scientific program on the campus at Urbana, June 10 and 11, to honor Professor William C. Rose who will retire to an emeritus status on the faculty at the end of the current academic year.

Paper Electrophoresis of Serum Proteins

The separation of serum proteins by electrophoresis using paper soaked in buffer as the transporting medium has become a simple and extremely useful method for analysis of the components albumin, alpha-, beta- and gamma-globulin fractions and their subdivisions. I. R. Mackay, W. Volwiler, and P. D. Goldsworthy (*J. Clin. Invest.* **33,** *855 (1954)*) have contributed a highly critical analysis of the method as applied to normal serum and to that obtained from patients with various disease states in which abnormalities of the serum proteins were to be expected. In many instances comparisons were made between the paper method and "free" electrophoresis using the Tiselius apparatus.

An accurate description is given of the method, the type of filter paper and buffer, the intensity and stability of the current,

the dye used for developing the color on the paper after electrophoresis and the methods for photometric measurement of the intensity of staining which is presumably an indication of the protein concentration. Multiple analyses of a single serum gave highly reproducible results with but small standard deviations in most instances.

It was pointed out that a poor correlation was obtained between paper and free electrophoresis on the serum from 2 patients with hypercholesterolemic xanthomatosis tuberosum. The discrepancies were greatest with the beta-globulin fraction and were considered, at least in part, to be due to a refraction of light in free electrophoresis by lipid components of this fraction.

The study can be recommended as a careful analysis of the various factors concerned in paper electrophoresis, a method which might be useful in field studies of protein nutrition and other nutritional problems.

Planning Low Sodium Meals

"Planning Low Sodium Meals," a recent publication of The Nutrition Foundation, is a new edition of the booklet published originally by the Newton Health Department. The booklet was designed to help patients whose doctors have placed them on sodium restriction choose menus that are nutritious, appetizing, and low in sodium. It was prepared jointly by the Newton Nutrition Center, Newton-Wellesley Hospital, Newton Heart Demonstration Program, the Massachusetts Department of Public Health, and the Department of Nutrition of Harvard University whose work was sponsored in part by The Nutrition Foundation. This new edition, revised to include pertinent literature references to research on low sodium diets, may be obtained from The Nutrition Foundation, Inc., 99 Park Avenue, New York 16, New York. Price: Fifty cents per copy.

Infant Malnutrition from Placental Disorders

One of the little known or discussed causes of malnutrition in infants is that arising from abnormalities in the placenta. Three such infants have been described by W. Pick (*New Engl. J. Med.* **250,** *905 (1954)*).

Large areas of old infarction were found in one placenta which was small in size. The second showed an area of hyalinization and marked changes in the umbilical arteries. The third was not examined microscopically but was very small, " 'about the size of an orange'." Calculations led to the belief that the infants were full term when delivered, although as the authors point out these estimates are subject to considerable error. The infants weighed at birth 1.8, 2.2 and 1.6 kg., respectively. The first seemed in satisfactory health except for the severe undernutrition. The second and third infants were quite ill, the second not being expected to live. All 3 did live, however, the first beginning to eat well immediately, the second after forty-eight hours, and the third on the third day. All 3 gained weight, strength and length rapidly, much more so than similarly sized premature infants, so that between 4 and 6 months of age they were indistinguishable from full term infants not undernourished. This is in contrast to the usual premature infant who doesn't regain his normal weight for four years in the case of males and for from nine to twelve months in the case of females.

These 3 infants, presumably born at full term but with the weight of markedly premature infants, demonstrate that if the undernutrition in this case is due to damage to the placenta and is not severe enough to be incompatible with life, the infants will begin soon to eat voraciously and rapidly to gain weight and strength so that within a short time they are indistinguishable from full term infants.

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The Nutrition Foundation was organized by food and related manufacturers in December, 1941, as a sincere expression of their interest in scientific progress and human health. The basic purposes of the Foundation are: (1) the development of a comprehensive program of fundamental research, providing basic information in the science of nutrition; and (2) the support of educational measures that will assist in making the science of nutrition effective in the lives of present and future generations.

The publication of NUTRITION REVIEWS has been undertaken to enable professionally trained people to keep abreast of current progress and to have available an unbiased, authoritative review of the current research literature in the science of nutrition.

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